Grey matter pathology in multiple sclerosis

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Chapter 1

General introduction

(Partly adapted from Kooi & Geurts, 2013; Book chapter in: "Handboek multiple sclerose")

1.1 Multiple sclerosis

Multiple sclerosis (MS) is the most common neurological disease among young adults and worldwide this debilitating disorder affects ~2.5 million individuals.[1,2] Remarkably, although MS occurs globally, the prevalence varies geographically and the risk is greatly influenced by latitude with the lowest prevalence near the equator and the highest in northern Europe, southern Australia and North America.[3,4] Clinically, MS usually starts in the third or fourth decade of life and has a highly variable disease course.[3-5] The majority of the MS patients (~80%) experience a relapsing-remitting disease course (termed relapsing-remitting MS (RRMS)), where reversible periods of neurological disability are interspersed by clinically silent episodes (Fig. 1). RRMS occurs twice as often in females than in males. Initial symptoms in these patients can be unilateral visual loss (optic neuritis), diplopia, ataxia, sensory disturbances, limb weakness, neurogenic bladder and bowel problems. Furthermore, many of the patients experience an unexplained fatigue. Approximately 65% of the RRMS patients enter the secondary progressive disease phase after a certain period of time, characterised by a gradual neurological deterioration (Fig. 1). A minority of the MS patients (10-15%) is characterised by a gradual decline in neurological disability from disease onset. This is the so-called primary progressive form of MS (PPMS).[6] In contrast to RRMS, PPMS has an equal incidence in males and females.

1.2 Genetics

Up until now, genome-wide association studies have clearly indicated several risk alleles for MS susceptibility.[7-11] Among these are single-nucleotide polymorphisms in the major histocompatibility complex (MHC) II region, interleukin (IL) 7 receptor α chain, CD6, IRF8 and TNFRSF1A. Without any exception, all MS susceptibility genes are involved in the immune system, with HLA-DRB1 as the most important MS susceptibility locus, increasing the risk with (only) 3-fold. Monozygotic twins have been shown to have an approximate concordance rate for MS of 30%,[12,13] where the MS discordance in monozygotic may not be explained by genetic, epigenetic, or transcriptomic differences,[14] suggesting an important role for environmental factors in the development of MS.

1.3 Environmental factors

For decades several environmental factors have been linked to MS among which are infections,[15,16] vitamin D deficiency,[17,18] sunlight exposure,[17] smoking[18,19] and hygiene.[20] The latitude gradient seen for MS prevalence is possibly the most striking example of how the environment may influence the risk for developing MS.[17] In addition, it is intriguing to see that migration from high-risk to low-risk regions in childhood is associated with a reduced risk for developing MS.[21-23] The most clearly associated infectious agent in MS is the Epstein-Barr virus (EBV). For example, it has been shown that >99% of the patients with MS are seropositive for EBV compared to 90-95% EBV seropositivity among controls.[15,24,25] Additionally, it has been shown that the risk for developing MS after EBV infection increases strongly[26] and people who have experienced infectious mononucleosis have a significantly increased risk for developing MS.[27,28] However, despite compelling epidemiological evidence for a link between EBV infection and MS, no direct role have been elucidated until now.

1.4 Neuropathology

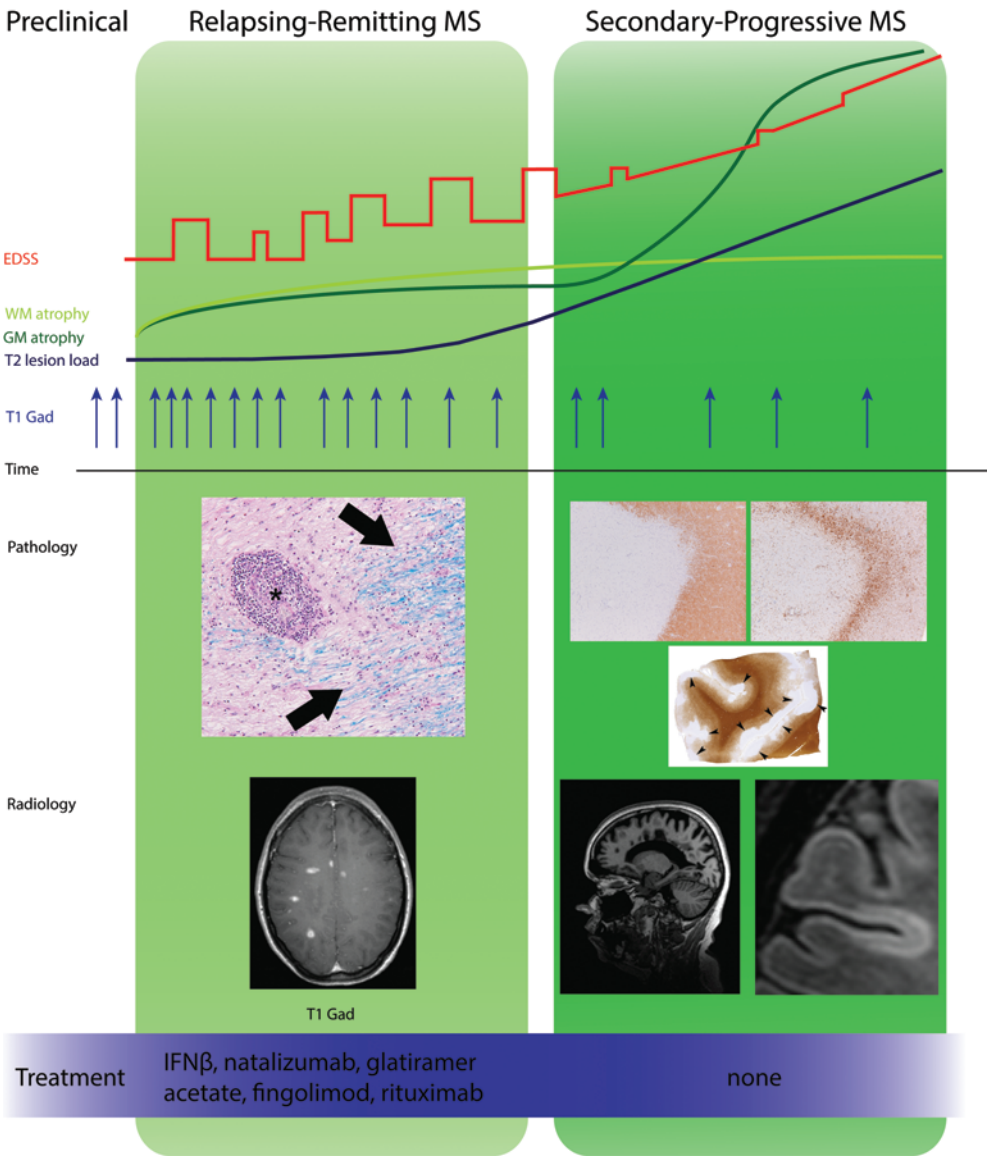

Figure 1. Schematic overview of clinical and pathological evolution of multiple sclerosis (MS). In the first stage of the disease (left box), inflammatory events mainly dominate. E.g. blood-brain barrier damage is a prominent feature as indicated by the presence of T1 gadolinium contrast enhancing lesions. In pathology, these lesions are characterised by perivascular cuffs (*) and inflammatory demyelination (arrows). In this stage of the disease several antiinflammatory drugs are effective. As the disease progresses (right box), grey matter (GM) pathology become much more prominent. Especially, GM atrophy rate accelerates rapidly during disease progression (dark green line; left magnetic resonance (MR) image). Furthermore, cortical lesions (CLs) are abundantly present in this phase of the disease (arrowheads). A major challenge will be to visualise these CLs with MR imaging, e.g. by high field MRI (right MR image; 7T 3D-MP-FLAIR, Courtesy of P.R. Luijten, Imaging Division UMC Utrecht and W.L. de Graaf, VUMC Amsterdam). White matter lesions become less inflammatory and are often mainly characterised by a slowly expanding (or so-called chronic active) process (upper two histopathology images).

MS is considered to be a chronic inflammatory demyelinating disease of the human central nervous system (CNS), characterised by focal regions of demyelination and axonal

loss throughout the brain and spinal cord.[29] Although heterogeneity has been proposed regarding the formation of white matter (WM) lesions[30] this has not been confirmed by a post-mortem study by our group.[31] In our group, a distinction is made between four different stages in WM lesion formation (Fig. 2).[32] Possibly, WM lesion formation starts with a cluster of activated microglia.[32] In this stage, there is no loss of myelin (yet) and this is called a 'preactive lesion' (Fig. 2A,B). It should be noted that, although plausible,[33] there is no final proof that these microglia clusters transit into a full-blown active WM MS lesions. The second stage in which we differentiate is the so-called 'active WM lesion' (Fig. 2C,D) characterised by the abundant presence of macrophages with intracellular myelin degradation products (Fig. 2D). At this stage, macrophages derived from blood-born monocytes became indistinguishable from macrophages derived of the CNS' endogenous microglia pool. Although in a lesser extent than macrophages, lymphocytes are also present in active WM lesions, with $CD8^+$ cytotoxic T-cells by far outnumbering $CD4^+$ T-helper cells or B-cells.[34-36] Interestingly, it has been shown that in particular $CD8^+$ cytotoxic T-cells in active MS lesions share antigen specificity[37] and even may persist as clonal expansions in the cerebrospinal fluid and blood after more than five years.[38] After active demyelination of a certain area in the CNS, lesions become chronically active, meaning that macrophages/microglia and lymphocytes mainly disappear from the center of the lesion and that a slow demyelinating process is continuing at the edges of the lesion, a so-called 'chronic active WM lesion' (Fig. 2E,F). Finally, the (multiple) demyelinated areas in the WM will be completely filled up with astrocytes yielding (sclerotic) scars in the CNS, these areas are called 'inactive WM lesions' (Fig. 2G,H).

1.5 Grey matter pathology in a 'white matter disease'

MS has classically been considered to be a WM disease, however, evidence is rapidly accumulating that the grey matter (GM) is also heavily affected. Demyelination of the GM have already been acknowledged for a long time,[39-41] but difficulties with the visualisation of GM lesions with standard histological staining techniques distracted MS investigators into the more striking WM pathology research field. By the end of the 20th century the attention for GM involvement in MS revived[42] and up until now the interest in GM involvement in MS is still rapidly increasing.

Based on their topological distribution cortical lesions (CLs) can be divided into mixed GM-WM lesions (type I lesions; see Fig. 3A) and intracortical lesions (type II, III; see Fig. 3B-C resp.).[43] Type II lesions are often small and perivascular and entirely located within the cerebral cortex (Fig. 3B).[43] Type III lesions extend from the pial surface and mostly stop at cortical layer 3 or 4 and often extend over multiple adjacent gyri (Fig. 3C).[43] Type IV lesions also extend from the cortical surface, but cover the entire width of the cerebral cortex (not shown).[43] While CL type I and II can be found at all disease stages,[44] subpial lesions (type III mainly) are most commonly seen in patients with disease progression.[44] Remarkably, CLs in the post-mortem setting are characterised by no lymphocyte influx,[45,46] a lack of complement activation[47] and an intact blood-brain barrier.[48] As type I lesion are contiguous with WM lesions, these lesions are most likely to be result of an inflammatory WM lesion continuing into the cortical GM.[42,45] Therefore, type I lesions, in contrast to subpial lesions, are also more inflammatory.[42,45] Whether the profound non-inflammatory character of subpial lesions in the post-mortem setting (and hence after a relatively long disease duration) is the result of an antecedent inflammatory process, is not elucidated yet. A

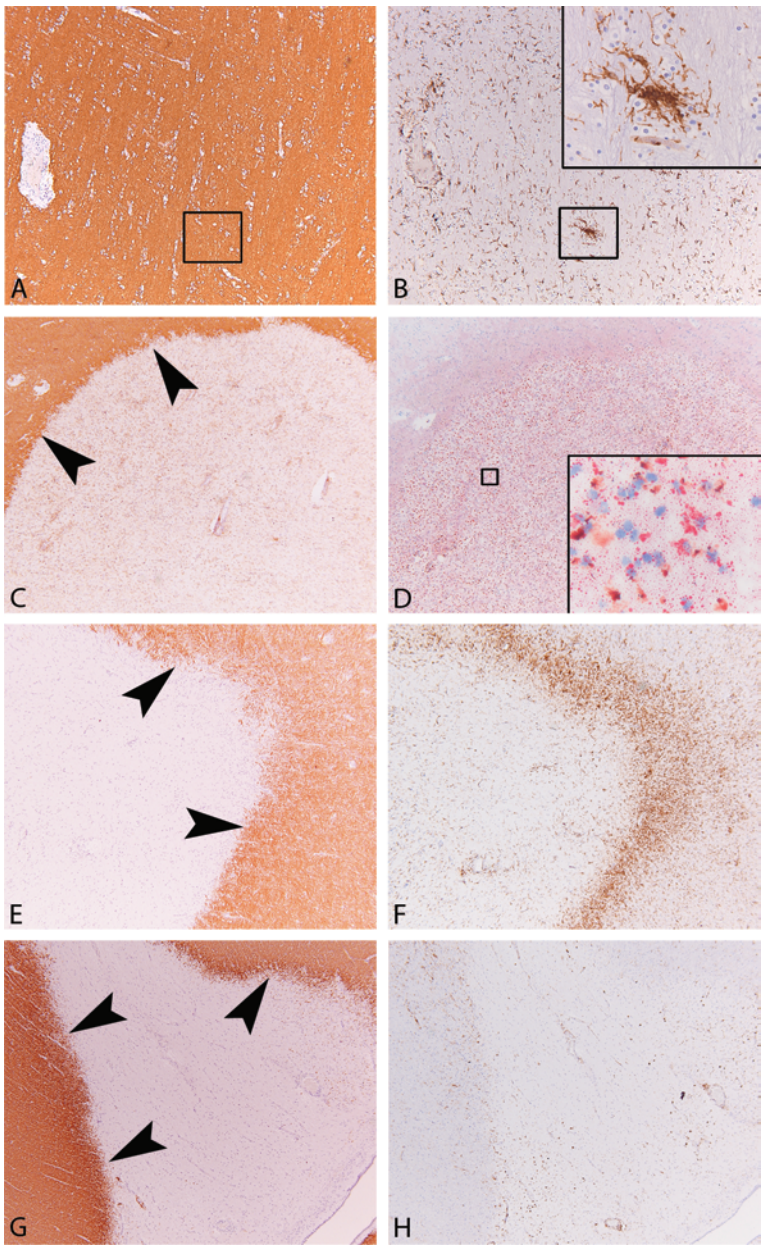

Figure 2. Staging of white matter (WM) multiple sclerosis (MS) lesions. Left panel (**A,C,E,G**) show immunohistochemical staining for myelin (proteolipid protein). Right panel (**B,F,H**) show immunohistochemical staining microglia/macrophages (human leukocyte common antigen DR, HLA DR), except figure D which shows a oil-red O staining (neutral lipids; myelin degradation products). (**A,B**) A cluster of microglia in normal-appearing white matter is thought to be the first lesion stage, this is a so-called preactive lesion. (**C,D**) A preactive lesions may become, under certain circumstances, a full-blown, active WM lesion which is characterised by a large number of actively demyelinating macrophages. (**E,F**) As the lesions progresses they become slowly expanding WM lesions, characterised by activated microglia at the edges of lesions. (**G,H**) Finally, the lesions become old sclerotic plaques without inflammatory activity.

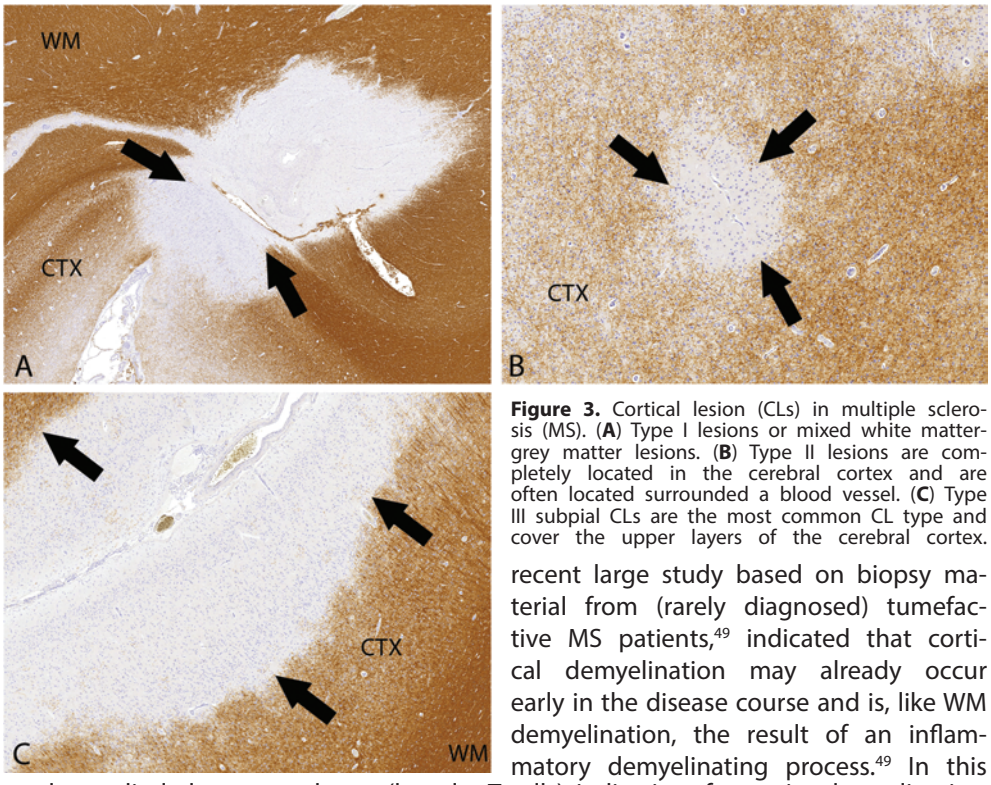

Figure 3. Cortical lesion (CLs) in multiple sclerosis (MS). (**A**) Type I lesions or mixed white matter-grey matter lesions. (**B**) Type II lesions are completely located in the cerebral cortex and are often located surrounded a blood vessel. (**C**) Type III subpial CLs are the most common CL type and cover the upper layers of the cerebral cortex.

recent large study based on biopsy material from (rarely diagnosed) tumefactive MS patients,[49] indicated that cortical demyelination may already occur early in the disease course and is, like WM demyelination, the result of an inflammatory demyelinating process.[49] In this study, myelin-laden macrophages (but also T-cells), indicative of an active demyelinating process, were found in a subset of the CLs and were most commonly in mixed GM-WM lesions (64%; 25 out of 39) and found in only 15% (4 out of 26) of the subpial lesions.[49] So, this study indicates that at least in a subset of the MS patients, cortical demyelination may occur on a background of inflammation. Whether this mechanism is responsible for the extensive subpial cortical demyelination, as can be found in MS disease progression, or rely on different mechanisms remains to be determined.

Currently, there is overwhelming evidence that virtually all GM structures can be affected by demyelination, including the (hypo)thalamus,[50-52] basal ganglia,[51,52] substantia nigra,[51] hippocampus[52-55] and cerebellar cortex.[56] The underlying pathogenic mechanisms of GM lesion formation are still not elucidated. This is possibly majorly handicapped by their low conspicuity on magnetic resonance (MR) images,[57,58] making the investigation of CL formation in time and space difficult. Despite these pitfalls, a lot of knowledge has been generated with MR imaging (MRI) techniques regarding the involvement of GM pathology in MS, not least with thanks to the use of newer MRI techniques.[59] For example, by making use of the 3D double inversion recovery (3D DIR) MR sequence it was shown that CL numbers increase over time[60-62], is indeed (as already suggested from pathology studies[44]) more common in disease progression[63] and is associated with clinical disability, brain atrophy and cognitive impairment.[61-65] Other studies clearly showed that GM atrophy rapidly accelerates during disease progression (Fig. 1) and that this atrophy, in contrast to WM pathologic changes, underlies the neurologic disability in MS.[66,67] It still has to be determined whether these accelerated GM atrophy is due to increased demyelination

of GM in progressive MS. At least in the short term, as reflected by work on marmoset experimental autoimmune encephalomyelitis (EAE), demyelination is not the explanatory factor underlying cortical atrophy.[68]

1.6 Neurodegeneration

MS is considered to be a putative autoimmune disorder with adaptive immune responses against CNS' auto-antigens.[69-75] These (auto)immune responses can be successfully repressed with the currently available antiinflammatory drugs (e.g. IFNβ, glatiramer acetate, natalizumab and fingolimod). A lot of the current knowledge about the (auto) immune aspects of the disease, as well as the basis for the current treatments for RRMS, is (primarily) based on EAE work.[76-78] However, the therapeutics may in the best case slow down disease progression, but none of them have been proven to halt or reverse disease progression in MS patients.[79,80] Remarkably, inflammatory brain lesions as seen on MR images correlate only very weakly with clinical disability.[81-83] However, it has been clearly shown that active WM lesions contain more than 11,000 transected axons per mm^3 and chronic active WM lesions contain more than 3000 transected axons per mm^3 at the edges of their lesions[84] indicating that axonal damage is highly correlated with the degree of inflammation.[84,85] Furthermore, it is widely accepted that axonal damage and neurodegeneration are the major causes of (irreversible) neurological disability in MS. However, especially in the beginning of the disease, it seems that the brain is able to compensate for the widely occurring axonal loss.[86-88] During disease progression, an increase in irreversible neurologic disability and GM atrophy occurs with a strong reduction of WM pathologic changes suggesting a more prominent role for GM damage in MS disease progression (Fig. 1).[66,67,89] Therefore, this underscores the need for research which may yield knowledge about the mechanisms underlying MS disease progression, and especially the increased GM pathology as observed in the progressive phase. This knowledge may pave the way for the development of new effective treatment options in this, for the neurologist and especially MS patient, desperate stage of the disease.

1.7 Aims and outline of thesis

Since MS has been regarded as a pure WM disease for more than a century now, only relatively little is known about GM pathology in MS. Therefore, **the aim of this thesis was to gain insight into GM pathology in MS**, which may lead to a better understanding of the possible underlying mechanisms and their clinical significance and it may generate hints for future therapeutic interventions to address GM damage and underlying clinical disability in MS.

In **chapter 2.1**, we investigated possible associations between meningeal inflammation (globally and regionally) with the presence and extent of subpial cortical demyelination. In **chapter 2.2**, we report the presence of myelin products in the meninges and perivascular spaces of MS patients and provide comparisons with other (demyelinating) neurodegenerative disorders. Then, **chapter 2.3**, is dedicated to the investigation of the clinical significance of MS CLs, and especially CLs with rims of activated microglia, which seem to predispose for a worse disease course.

As the acetylcholine neurotransmitter system is essential in learning and memory function, and a significant proportion of the MS patients suffer from cognitive (memory) problems, as well as from severe hippocampal pathology, we investigated in **chapter**

3.1 whether the acetylcholine neurotransmitter system in the MS hippocampus might be abnormal in pathologic and neurochemical terms. **Chapter 3.2** features a scientific correspondence based on our data to a recently published clinical trial, where MS patients were treated with acetylcholinesterase inhibitors in order to improve their memory function. In **chapter 4.1**, microarray-based global gene expression analyses were performed on CLs, normal-appearing cortex and control cortex in order to study genes possibly involved in CL pathogenesis. Additionally, tissue samples were screened for the presence of Epstein-Barr virus, as this virus was hotly debated as a potential pathogenetic factor in MS cortical lesion formation over the past few years. In **chapter 4.2**, microRNA and their predicted mRNA targets in CLs, chronic active WM lesions, normal-appearing WM and control WM were investigated by using microarray-based technology.

As only a small proportion of the CLs can be detected by conventional MR imaging techniques, we investigated in **chapter 5.1** whether CLs that are visible on conventional MR images differ from MRI-invisible CLs in terms of underlying histopathology and quantitative MRI measures. In **chapter 5.2**, the widely used 3D DIR MR sequence for CL detections, was formally validated for its sensitivity and specificity by making a side-by-side review of MR images with the corresponding gold-standard of histopathology. Finally, in **chapter 6** the results of the previous chapters are summarised and discussed and guidelines for future research are given.

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